

Heat shock induces enzymes of trehalose metabolism, trehalose accumulation, and thermotolerance in *Schizosaccharomyces pombe*, even in the presence of cycloheximide

Claudio De Virgilio, Urs Simmen, Thomas Hottiger*, Thomas Boller and Andres Wiemken

Department of Botany, University of Basel, Basel, Switzerland

Received 11 September 1990

Exponentially growing cells of the fission yeast, *Schizosaccharomyces pombe*, contained virtually no trehalose at 27°C but rapidly accumulated large quantities during heat shock at 40°C. Activities of trehalose-6-phosphate synthase and trehalase also increased upon heat shock. Thermotolerance of the cells, measured as survival at 52°C, increased in parallel to trehalose accumulation and decreased in parallel to the trehalose levels when cells were shifted back to 27°C. Trehalose levels, activities of enzymes of trehalose metabolism and thermotolerance strongly increased upon heat shock even in the presence of cycloheximide, indicating that none of these effects requires protein synthesis. The data support the hypothesis that trehalose acts as a thermoprotectant in *Schizosaccharomyces pombe*.

Trehalose; Heat shock; Thermotolerance; Trehalase; Trehalose-6-phosphate synthase; *Schizosaccharomyces pombe*

1. INTRODUCTION

Trehalose is a non-reducing disaccharide (1- α -D-glucopyranosyl-1,1- α -D-glucopyranoside) widely distributed in both prokaryotes and eukaryotes [1,2]. It occurs ubiquitously in fungi, particularly in their anhydrobyotic resting forms such as spores, conidia, sclerotia, etc. [1–7]. Trehalose is present in the cytosol [8] and is generally thought to function, together with glycogen, as the main storage carbohydrate of fungi [5,9]. However, as recently reviewed [6,7], several lines of evidence suggest that the main role of trehalose might not be to act as a carbon and energy reserve but rather as a protectant against physical stresses such as desiccation, heat and freezing.

Support for this notion comes from our work on the budding yeast, *Saccharomyces cerevisiae*: We manipulated the cellular pool of trehalose by a mild heat shock [10] which induces rapid accumulation of trehalose [11], and by the use of mutants impaired in the ras-adenylate cyclase pathway [12] which is involved in the regulation of trehalose metabolism of *Saccharomyces cerevisiae* [5,13]. We found a close correlation between trehalose levels and tolerance to heat and desiccation [10,12]. Resistance to freeze drying [14] and

to osmotic stress [15] has also been found to be correlated with the trehalose contents of *Saccharomyces cerevisiae*.

We became interested to examine if trehalose had a similar role in the fission yeast, *Schizosaccharomyces pombe*, which is known to differ profoundly in many respects from budding yeast at the cytological and molecular level [16]. In *S. pombe*, trehalose has hitherto been found only in the spores just before they reach maturity and enter into the dormant state [4], and the only enzyme of trehalose metabolism described so far is an acid trehalase specifically associated with the cell walls of the ascospores [3,4].

Here we report that trehalose accumulation in *S. pombe* is not a sporulation specific event as has been suggested [3] but can be rapidly induced, as in *Saccharomyces cerevisiae*, even in log-phase cultures by heat shock. Furthermore, we describe heat shock-induced activation of two key enzymes of trehalose metabolism, both hitherto unknown from *Schizosaccharomyces pombe*, namely trehalose-6-phosphate synthase and a trehalase with neutral pH optimum. Finally, we demonstrate that the trehalose content of the cells and their thermotolerance increase upon heat shock in a closely parallel way both in the absence and in the presence of cycloheximide, suggesting that trehalose accumulation but not protein synthesis is required for acquired thermotolerance, and corroborating the concept of the role of trehalose as a general stress protectant previously proposed for *Saccharomyces cerevisiae* [8,11,12].

Correspondence address: A. Wiemken, Botanisches Institut, Hebelstrasse 1, CH-4056 Basel, Switzerland

*Present address: Department of Chemical Engineering, Caltech, Pasadena, CA 91125, USA

2. MATERIALS AND METHODS

2.1. Organism and culture conditions

Schizosaccharomyces pombe wild type strain 972h (received from Prof. P. Nurse, Oxford University, UK) was kept as stock culture on YPDA (yeast extract 1%, bacto-peptone 2%, glucose 2%, agar 2%) and grown at 27°C on a shaker (140 rpm) in liquid YPD-medium (as above but no agar). For labeling experiments, Edinburgh Minimal Medium No. 2, EMM2 [17] was used. Well adapted log-phase cultures (at least 5 generations of exponential growth) at a density of $0.5-1 \times 10^7$ cells per ml were used for the heat shock experiments. For the transfer into a glucose-deficient medium, cells were rapidly harvested on Whatman GF/C filters, washed with two portions of prewarmed glucose-free YPD-medium and suspended in a third portion of the same medium.

2.2. Heat shock conditions and analysis of thermotolerance

Cultures (100 ml in 1000-ml culture flasks) were subjected to heat shock by transfer into a shaking water bath at 40°C and relieved from heat shock by transfer to a water bath at 27°C. To determine thermotolerance, aliquots of the culture (1 ml) were transferred to prewarmed 15 ml glass tubes, incubated for 8 min at 52°C, cooled in an ice bath, and plated on YPDA. Colonies were counted after 2 days at 27°C, and the % survival was assessed by comparison with controls not subjected to the 52°C treatment (100%). The number of colonies in such controls after heat shock to 40°C and/or after cycloheximide treatment was similar to that of untreated controls, indicating that these treatments did not decrease viability.

2.3. Enzyme extraction and assays

Cells containing ca 15 mg of protein were harvested by filtration (Whatman GF/C), washed three times with 5 ml of ice cold water and suspended in 0.6 ml of 0.2 M Tricine (Na^+) buffer, pH 7.0, in a 1.5 ml Eppendorf tube. For cell disruption the filters were removed from the tubes, 1.7 g glass beads (diameter 0.5 mm) were added and the resulting slurry was vigorously shaken at 0–4°C until at least 95% of the cells were disrupted. The homogenates were desalted on a Sephadex G-25 column (bed volume 2 ml) and used immediately for the enzyme assays. The assay of trehalase was performed in 50 mM Mes(K^+), pH 6.0 (this is the pH optimum), 100 mM trehalose, 0.2 mM MnCl_2 and enzyme extract in a total volume of 400 μl . After incubation for 10–30 min at 35°C the reaction was stopped in a boiling water bath (3 min). After cooling, assay mixtures were centrifuged at $3000 \times g$ for 10 min. Glucose was determined in the supernatant using the GOD-test kit from Boehringer, Mannheim, FRG.

Trehalose-6-phosphate synthase was measured by a coupled enzyme assay essentially as described [11]; however incubation was at 50°C (this is the temperature optimum) in a total volume of 240 μl . The reaction was stopped in a boiling water bath (5 min). After cooling, assay mixtures were centrifuged at $2000 \times g$ for 10 min, and the UDP formed was measured enzymatically in the supernatant as described [11].

2.4. Determination of trehalose and protein

Trehalose was extracted by trichloroacetic acid and determined by the anthrone procedure as described [11]. In all types of experiments, its identity was verified by thin layer chromatography [11]. Protein was determined as before [11] using bovine serum albumin as a standard.

2.5. Chemicals and enzymes

Chemicals and enzymes were obtained from Sigma and L-[^{35}S]methionine (42 MBq nmol $^{-1}$) from NEN-Dupont, Boston, MA.

3. RESULTS AND DISCUSSION

S. pombe cells growing in mid-log-phase at 27°C contained only traces of trehalose. However, when the cultures were shifted to 40°C the cells rapidly ac-

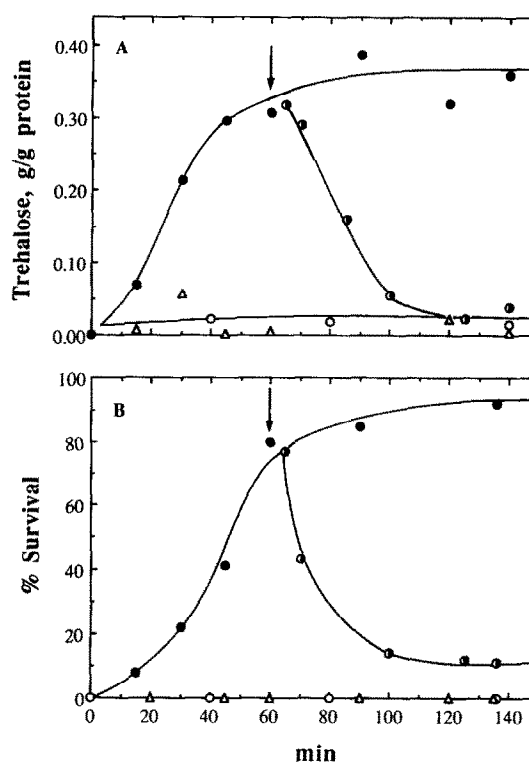


Fig. 1. Trehalose levels (A) and thermotolerance, assessed as survival at 52°C for 8 min (B) of *Schizosaccharomyces pombe* during and after a heat shock. Log-phase cultures were shifted from 27°C to 40°C on media with glucose (●) or without glucose (△) at 0 min. Part of the culture on glucose (●) was shifted back to 27°C after 60 min (arrow); control cells were maintained with glucose at 27°C (○).

cumulated trehalose in large amounts and rapidly lost it again after shifting the cultures back to 27°C (Fig. 1A). The presence of glucose in the medium was a prerequisite for the accumulation of trehalose during the heat shock (Fig. 1A). Remarkably, thermotolerance of the cultures was found to change in parallel with the trehalose content of the cells (Fig. 1B). The presence of glucose in the medium was also necessary for heat shock induced thermotolerance.

Both trehalose accumulation and induction of trehalase have been reported to be sporulation specific events in *S. pombe* [4]. It was surprising therefore to find that trehalose accumulation and mobilization started almost instantaneously upon heat shock and after relief from it, respectively. We tested the heat shocked cells for trehalose metabolizing enzymes and found that activities of trehalase and trehalose-6-phosphate synthase strongly increased during heat shock, the former with a lag of 30–60 min (Fig. 2A) and the latter with a lag of only 10–15 min (Fig. 2B). The trehalase had a pH optimum of pH 6.0 (data not shown). After relief from the heat shock, the activities of both enzymes remained more or less constant (Fig. 2). The changes of enzyme activities did not accurately reflect the rates of accumulation and mobilization of

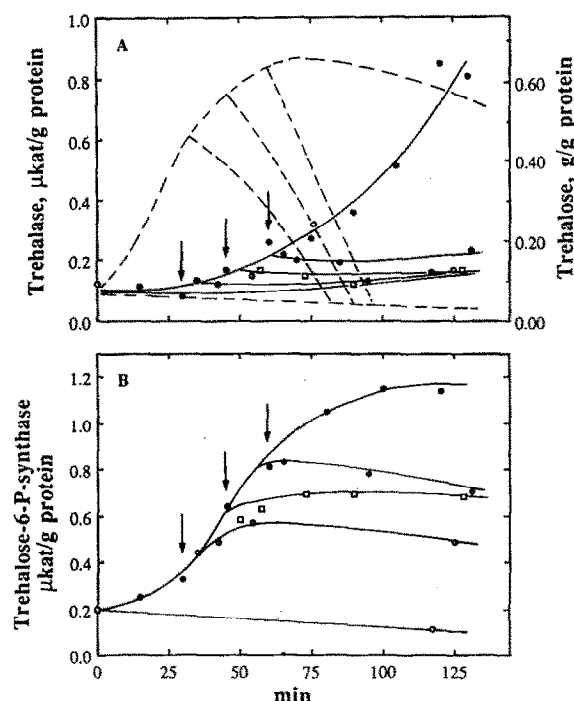


Fig. 2. Activities of trehalase (A) and trehalose-6-P-synthase (B) in *Schizosaccharomyces pombe* during and after a heat shock. Log-phase cultures were shifted from 27°C to 40°C at 0 min (●). Part of the cultures were shifted back to 27°C at the times indicated by arrows, i.e. after 30 min (○), 45 min (□), and 60 min (◇). Control cells (○) were maintained at 27°C. For comparison, the trehalose levels determined in the same experiment are shown by the dashed lines in (A).

trehalose in the cultures: during heat shock, trehalose was accumulated at maximal rate between 15 and 30 min (Fig. 1, Fig. 2A), much before the highest activity of trehalose-6-phosphate synthase was measured (Fig. 2B). Similarly, after relief from heat shock, the trehalose pool of the cells declined rapidly already before the activity of trehalase was high (Fig. 2A).

The rapid response of the trehalose pool to heat shock and the inconsistencies between the enzyme activities measured in vitro and the changes of the trehalose pool in vivo indicated that the enzymes might be present constitutively and regulated at a posttranslational level, as described for trehalase in other fungi [5]. We tested this by adding cycloheximide (50 μg/ml) added 5 min before starting the heat shock. The accumulation of trehalose (Fig. 3A) and the acquisition of thermotolerance (Fig. 3B) as well as the increase in the activities of the two enzymes (Fig. 3C,D) were barely affected by the cycloheximide treatment. The same results were obtained when larger amounts of cycloheximide were added (up to 100 μg/ml). In control experiments (incubation of cultures containing 10⁷ cells per ml in EMM2 medium with 220 kBq [³⁵S]methionine for 60 min, starting 3 min after initiation of the heat shock), cycloheximide inhibited the incorporation of [³⁵S]methionine into TCA-precipitable material by more than 90% (data not shown). At 27°C, cycloheximide arrested growth of the cultures (data not shown) but had no effect on the trehalose levels in the cells (Fig. 3A).

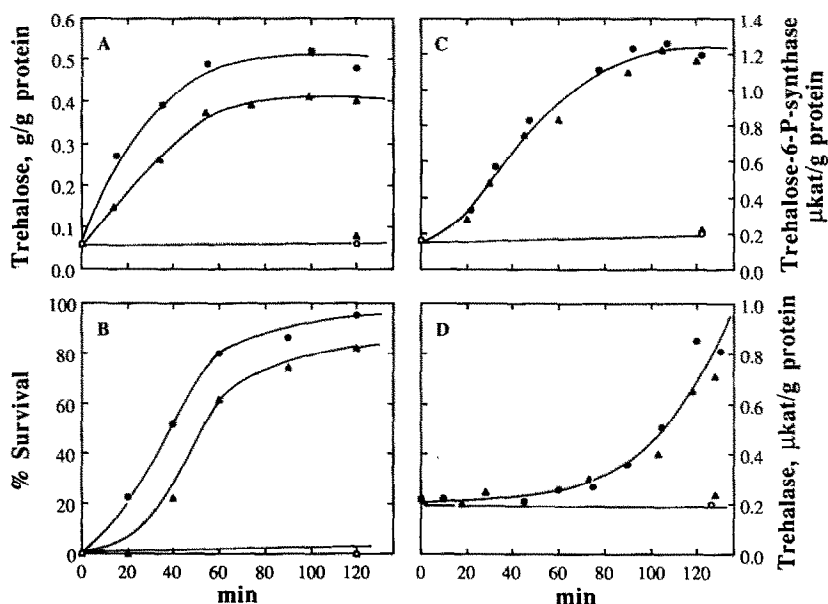


Fig. 3. Effect of cycloheximide on the heat-shock induced increases of trehalose levels (A), thermotolerance, assessed as survival at 52°C for 8 min (B), and activities of trehalose-6-phosphate synthase (C) and trehalase (D). Log-phase cells of *Schizosaccharomyces pombe* were shifted from 27°C to 40°C at 0 min in the absence of cycloheximide (●) or in the presence of cycloheximide (50 μg·ml⁻¹), added 5 min before initiation of the heat shock (▲). Controls were maintained at 27°C in the absence (○) or presence (△) of cycloheximide.

These results indicate that in *S. pombe* the trehalose metabolising enzymes are constitutively expressed and subject to posttranslational regulation the nature of which we are currently investigating. The close correlation between acquisition of thermotolerance and trehalose accumulation during a heat shock, even in the presence of cycloheximide, supports the concept that trehalose plays a role as a thermoprotectant [7,10,12] and casts some doubts on the importance of heat shock proteins for thermoprotection.

Acknowledgements: We thank Karin Flükiger for technical assistance and Walter Bell and Joachim Müller for discussions and helpful suggestions. This work was supported by the Swiss National Science Foundation.

REFERENCES

- [1] Crowe, J.H., Crowe, L.M. and Chapman, D. (1984) *Science* 223, 701-703.
- [2] Elbein, A.D. (1974) *Adv. Carbohydr. Chem. Biochem.* 30, 227-256.
- [3] Inoue, H. and Shimoda, C. (1981) *Arch. Microbiol.* 129, 19-22.
- [4] Inoue, H. and Shimoda, C. (1981) *Mol. Gen. Genet.* 183, 32-36.
- [5] Thevelein, J.M. (1984) *Microbiol. Rev.* 48, 42-59.
- [6] Van Laere, A. (1989) *FEMS Microbiol. Rev.* 63, 201-210.
- [7] Wiemken, A. (1990) *A. Leeuwenhoek Int. J. Gen. Microbiol.* (in press)
- [8] Keller, F., Schellenberg, M. and Wiemken, A. (1982) *Arch. Microbiol.* 131, 298-301.
- [9] Lillie, S.H. and Pringle, J.R. (1980) *J. Bacteriol.* 143, 1384-1394.
- [10] Hottiger, T., Boller, T. and Wiemken, A. (1987) *FEBS Lett.* 220, 113-115.
- [11] Hottiger, T., Schmutz, P. and Wiemken, A. (1987) *J. Bacteriol.* 169, 5518-5522.
- [12] Hottiger, T., Boller, T. and Wiemken, A. (1989) *FEBS Lett.* 255, 431-434.
- [13] Thevelein, J.M. (1988) *Exp. Mycol.* 12, 1-12.
- [14] Gadd, G.M., Chalmers, K. and Reed, R.H. (1987) *FEMS Microbiol. Lett.* 48, 249-254.
- [15] Mackenzie, K.F., Singh, K.K. and Brown, A.D. (1988) *J. Gen. Microbiol.* 134, 1661-1666.
- [16] Russell, P. and Nurse, P. (1986) *Cell* 45, 781-782.
- [17] Fantes, P.A. and Nurse, P. (1977) *Exp. Cell Res.* 107, 377-386.